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Information Sheet

NEW PEROXIDASE TEST PROCEDURE FOR DEHYDRATED POTATOES TO INDICATE ADEQUACY OF BLANCHING

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Lack of detailed information on the amount of blanching that is necessary to insure desirable storage characteristics in dehydrated white potatoes has forced purchasing agencies to require a wide margin of safety. Results of extensive research have now shown that this margin can be reduced safely, and that the use of reduced blanching lessens the tendency of the rehydrated product to be mushy. This tendency was particularly marked in potatoes dehydrated in 1943. The purpose here is to describe a new test that is especially adapted to the determination of peroxidase activity in potatoes prepared in accordance with the lower blanching requirement.

The new test is similar to tests that have been used but minimizes the subjective factor in color estimation by the use of permanent standards to determine whether the color intensity exceeds a specified tolerance. It is based on the intensity of color developed in solution in a specified time by the enzymic oxidation of guaiacol. The estimation is made by visual comparison of a solution with a set of standards described below. In contrast to methods formerly used for inspection, the color developed on the solid pieces is disregarded. Those who have used other peroxidase methods will note that the test conditions are more favorable for the activity of peroxidase and therefore more color will generally develop, both in solution and on the solid pieces, than under the conditions of the other tests. (See U. S. Dept. Agr. Misc. Pub. 540, Vegetable and Fruit Dehydration, pages 36 and 147, for further discussion of blanching and testing for adequacy of blanching.)

Reagents: (Use A.C.S., U.S.P., N.F., or equivalent grade chemicals.)

10 percent guaiacol in 95 percent undenatured ethyl alcohol
30 percent hydrogen peroxide
(Because hydrogen peroxide decomposes at elevated temperatures and in the presence of traces of impurities, it is best to keep the stock bottle of 30 percent hydrogen peroxide in a cool place and to pour a small amount into a small dropping bottle for daily use.)
5M ammonium acetate (386 gm. of $(NH_4)C_2H_3O_2$ dissolved in sufficient distilled water to make one liter).

Apparatus: (Laboratories running large numbers of tests may require larger supplies of certain items than are indicated below.)

1 comparator block
1 set of color standards (color scores 0, 50, 100, 200, and 300)
1 extra standard of color score 50
6 1-liter beakers
24 18x150-mm. pyrex test tubes
3 10-ml. pipettes
6 65-mm. funnels

1 test-tube rack
1 box of 15-cm. fluted filter paper, Whatman No. 12 or similar grade
6 stirring rods (glass rods, aluminum or plastic spoons are satisfactory--do not use iron)
Containers for the ammonium acetate, guaiacol, and hydrogen peroxide (The hydrogen peroxide may be placed in a small (30 or 60 ml.) dropping bottle--see reagents)
1 liter graduated cylinder
1 thermometer, 0 to 110° C.

Color standards: The standards contain potassium dichromate and cobaltous nitrate in the ratio of 1 part of $K_2Cr_2O_7$ to 65 parts of $Co(NO_3)_2 \cdot 6H_2O$. The standard corresponding to the presently recommended tolerance 1/ contains 0.040 gm. of $K_2Cr_2O_7$ and 2.60 gm. of $Co(NO_3)_2 \cdot 6H_2O$ per liter of solution. This solution is assigned a color score of 50. A score of 100, then, is represented by a solution of twice this concentration and an arbitrary color scale is thus set up. It is desirable in estimating the peroxidase activity of samples to have available color standards of scores 0, 50, 100, 200, and 300. It is convenient to make up the most concentrated standard corresponding to a color value of 300 (0.24 gm. of $K_2Cr_2O_7$ and 15.6 gm. of $Co(NO_3)_2 \cdot 6H_2O$ per liter of solution) and to prepare the others by dilution as follows: 100 ml. of standard 300 diluted to 150 ml. gives standard 200; 100 ml. of standard 300 diluted to 300 ml. gives standard 100; and 100 ml. of standard 300 diluted to 600 ml. gives standard 50. The 0 standard is distilled water. Care must be taken in preparing the standard and in making the dilutions.

The standards should be dispensed in 18x150-mm. pyrex test tubes, which should be sealed to prevent evaporation. Comparisons are made by looking through the diameter of the tube, not the length. It is convenient to place the tubes in a comparator block (or a test-tube rack) with a white background.

Procedure:2/ 1. Weigh out a 50-gm. portion of each sample of dehydrated potatoes, place it in a liter beaker, and add 10 ml. of 5M ammonium acetate, followed by 540 ml. of distilled water. Stir thoroughly with a stirring rod and allow to rehydrate for 1 hour. The temperature of the distilled water and of the mixture during rehydration and reaction should be between 20 and 27° C.

2. After the hour of rehydration add 10 ml. of 10 percent guaiacol, stir, and add 6 drops (0.30 ml.) of 30 percent hydrogen peroxide (equivalent amounts of other concentrations may be used). Again stir thoroughly and allow to react for exactly 1 hour without agitation. During this reaction time protect the sample from sunlight and intense artificial light--e.g., by covering the beakers with a towel. Strong illumination causes fading of the color.

1/ This tolerance is lower than the maximum tolerance suggested in U. S. Dept. Agr. Misc. Pub. 540, to correspond with the safest practical margin of blanching on potatoes that may encounter severe transportation and storage conditions.

2/ This procedure differs from that presented in U. S. Dept. Agr. Misc. Pub. 540, page 150, only in minor detail and will give the same coloration in the solution as the one described there.

3. At the end of the hour of reaction time, stir thoroughly with a stirring rod. The contents of the beaker should be made to swirl in each direction several times until the color, if any, is uniformly distributed throughout the solution. Then filter a portion of the sample through fairly fast filter paper (e.g., Whatman No. 12 fluted paper) into an 18x150-mm. pyrex test tube and estimate the extent of coloration by comparison with the color standards. The filtration can be accomplished with a 65-mm. funnel supported by the test tube. The stirring, filtering, and comparison must be completed within 5 minutes, because the color has a tendency to fade.

Interpretation: The color score tolerance now recommended is 50. On this basis, samples that give a color score of 50 or less are considered to be satisfactorily blanched.

Discussion of Peroxidase Tests for Quality-Control Use at Discharge End of Blancher

As previously mentioned, the procedure and interpretation described above are to be used only on dehydrated potatoes. The test that the processor uses on freshly blanched potatoes may differ considerably from this test, and from tests used by other processors. As will be shown later, the correlation between any available test for undehydrated blanched potatoes and the new test for dehydrated potatoes will vary from plant to plant.

It is expected that the present blanching practice of most dehydrators will be adequate and that the dehydrator may wish to continue to use the quality-control test he now uses. If so, the adequacy of his method can be checked by peroxidase tests (new method) on dehydrated potatoes that barely passed or did not pass his quality-control test. Potatoes may be encountered, however, that will not withstand sufficient blanching to pass the processor's present quality-control peroxidase test. The following remarks may be of assistance to him in developing a new quality-control test:

The results of many tests at this Laboratory have shown that peroxidase activity remaining in potatoes after short blanching treatments is partially destroyed during dehydration. As expected, the destruction increased as the severity of the drying condition was increased, with respect to both temperature and duration. Also, other factors, such as variety and storage conditions of raw material, may affect the amount of destruction brought about by dehydration. For these reasons, the amount of destruction of peroxidase that will occur under different plant practices cannot be predicted. Therefore, all processors may not wish to employ exactly the same test at the discharge end of the blancher and in fact may find it expedient to vary the conditions with seasonal or varietal changes in raw material.

It is suggested that the operator concerned with the development of a processing-control test use a modification of the new peroxidase test if his present practice is found to be unsuitable. Since he will want to know as soon as possible whether to modify his blanch, his control test should require less time than the hour of reaction specified in the regular test. This condition can be met because of the higher peroxidase activity (if any) in the blanched

than in the corresponding blanched and dehydrated samples. For example, in a test carried out on a laboratory scale the following results were obtained:

Reaction time	Peroxidase score: Blanched only*	Peroxidase score: Blanched and dehydrated
15 min.	15	--
30 min.	150	--
60 min.	275	0

*The modified peroxidase procedure suggested below for use on undehydrated potatoes was used.

From such data the operator would conclude that potatoes from his blancher that give a peroxidase score of 150 or less with a 30-minute reaction time, or of about 15 (estimated) or less with a 15-minute reaction time, would readily pass the 50-score tolerance following dehydration under his plant conditions. Additional tests would show how high a score on the blanched material would correspond to a tolerance of 50 on the dehydrated material.

The operator may thus use a 15-minute reaction time (or other convenient time) and determine the color score on his blanched potatoes that, under his drying conditions, will enable him to meet the tolerance. (The dehydrated potatoes are submitted, of course, to the regular peroxidase test, which uses a 60-minute reaction time.) With data thus obtained and some experience, the operator will be able to select a reaction time and color-score tolerance for use on the blanched product that will give him a margin of safety to allow for minor variations in the operation of his equipment and in his raw stock. To assure a margin of safety he will select a tolerance for the blanched material that corresponds to a score well below 50 on the dehydrated material.

Modification of peroxidase tests to be used on blanched undehydrated potatoes: The peroxidase procedure proposed for use on the dehydrated samples may be used with certain modifications:

The reagents and apparatus are the same.

The size of sample and the amount of water used are different. In order to realize approximately the same dry weight of potatoes and volume of reaction mixture, use 250 gm. of blanched potato and 370 ml. of distilled water. Place these in a liter beaker and cool to about 25° C. by placing the beaker in flowing tap water. The ammonium acetate,[3/] guaiacol, and peroxide are then added and the regular procedure subsequently followed except that the reaction time may be varied to obtain the desired "end point".

Smaller-sized samples may be used, provided proportionate amounts of water and reagents are used. Such samples would be less representative but may be suitable for quality-control purposes.

3/ In this case it is unnecessary to add the ammonium acetate before the water is added.